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Effect of anthelmintics on the succinic dehydrogenase activity
of *Ascaris lumbricoides* f. *suis*

Działanie leków przeciwwrobaczych na aktywność dehydrogenazy
kwasu bursztynowego glisty *Ascaris lumbricoides* f. *suis*

The mechanism of the anthelmintic action is not still fully understood. Few experiments were especially designed for evaluation of the effect of anthelmintics on parasite enzymes (e. g. Krotov et Kac 1958).

The dehydrogenases are of great importance to *Ascaris lumbricoides* as for an endoparasitic organism living under conditions which are essentially anaërobie. Lipschitz et Gottschalk 1921 showed that pulp of *Ascaris megalcephala* reduced o-dinitrobenzene in the presence of the succinic or lactic acid. This process was inhibited by oxygen, urethane, propyl alcohol and methyl ketone. These authors established also the presence of lactic dehydrogenase in *Ascaris lumbricoides*. A glutamic dehydrogenase was also detected (Chance et Dirnhuber 1949).

The succinic dehydrogenase usually is a part of the cytochrome oxidase system which is universally distributed in the animal kingdom. Van Grembergen et al. 1949 postulated the presence of cytochrome oxidase in muscle pulp of *Ascaris* as the addition of paraphenylenediamine stimulated oxygen uptake. This oxydation, however, is not specific to cytochrome oxidase. *Ascaris* muscle has long been known to contain a pigment believed to be cytochrome C (Keilin 1925). Bueding et Charms 1952 challenged this identification, and showed that muscle contained at most only traces of enzymically active cytochrome C or cytochrome oxidase. These observations were confirmed in part by Rathbone 1955. Enough information is now at hand to indicate that the activation and reduction of oxygen by *Ascaris* muscle preparations is not catalyzed by the cytochrome oxidase system (Laser 1944, Bueding and others 1955). The succinoxidase system of *Ascaris* muscle is complex and still incompletely understood (Chin et Bueding 1954, Rathbone 1955).

These studies were partially supported by a grant from the Parasitological Committee of the Polish Academy of Sciences, Section II.

The action of anthelmintics on succinic dehydrogenase activity of helminths is insufficiently explored. K r o t o v 1958 reported that the dehydrogenase activity is inhibited by phenothiazine. It must be noted that only a few statements on the effect of anthelmintics on dehydrogenase activity are present in papers of O e l k e r s 1950, B r a n d 1952, P a r i b o k 1954 and others, though the question of anthelmintics action on biochemical processes of endoparasitic worms is considered to be of great importance (S k r j a b i n 1947, B r a n d 1952, P a r i b o k 1954).

Methods

Succinic dehydrogenase was prepared according to the modified procedure of Keilin et Hartree 1938. The adult worms *Ascaris lumbricoides* f. *suis* obtained from slaughter-house were transported to laboratory with intestine content in thermos flask. The worms were washed, placed in a container with craked ice, and then eviscerated. *Ascaris* muscle was ground with quartz sand and 0.05M phosphate buffer, pH 7.4, at 0°C. The muscle pulp was squeezed through cheesecloth, and the extract adjusted do pH 5.0 by trating with 0.2 acetate buffer, pH 4.4. The precipitate was collected by centrifugation and redissolved in 0.333M phosphate buffer, pH 7.4. The preparation was stored in the cold.

The dehydrogenase activity was determined by method of Bertho 1936 in 13-ml test tubes supplied with non-corrosive puncture needles instead of glass tubules. Nitrogen, purified from oxygen with 33% pyrogallol in concentrated potassium hydroxide, was transmitted through 10 ml of sample solution for 10 minutes.

Assay conditions: 5×10^{-5} M methylene blue, 3×10^{-3} M sodium succinate, 36.7×10^{-3} M phosphate buffer, pH 7.4, water bath at 38°C with deviations to 0.5°. Distilled water was added to control test tubes instead of tested drug solution.

The decolorization time was determined with accuracy of 1 min. using a standard solution of 90% decolorization; the decolorization time of control determinations was 18—27 min. The standard deviation of control activity determinations (accepted as 100.0) $s = \pm 1.64$, the standard error $e = \pm 0.193$.

The anthelmintic drugs were tested in concentrations 10^{-1} M — 10^{-4} M, or 0.0001% — 1.68%, in the case of drugs of unknown chemical constitution or composition. The decolorization time of sample with added anthelmintic solution, in relation to control sample accepted as 100.0%, was a measure of the succinic dehydrogenase activity. The activity of samples with decolorization time amounting to 6 hr. was settled as 0%. The significance of the difference was tested with t test (Fisher 1954). The drugs concentration reducing dehydrogenase activity to 50% was calculated according to the regression equation.

Example: I series of experiments (X. 1959)

Piperazine adipate

	Decolorization time	Control	Mean activity $\pm$ standard error
10^{-1} M	6 hr.	22 min.	0%
10^{-2} M	40, 43, 40, 42, 40, 40, 42, 41, 41 min.	22, 22, 22 min.	$53.7 \pm 0.54\%$
10^{-3} M	23, 23, 23, 22, 23, 23 min.	23, 23 min.	$100.8 \pm 0.84\%$
10^{-4} M	22, 23, 23 min.	23 min.	$101.5 \pm 1.68\%$

Results

Our investigations showed that all the inorganic and metalorganic anthelmintics examined inhibited the succinic dehydrogenase activity of *Ascaris lumbricoides* f. suis, viz., mercuric chloride (concentration reducing the activity to 50% 0.27×10^{-9} M), merthiolate (5.2×10^{-6} M), aluminium sulphate (3.2×10^{-3} M), sodium fluoride (18.6×10^{-3} M), potassium antimonyl tartrate (45.0×10^{-3} M), potassium iodide (95.5×10^{-3} M). It must be noted that mercury compounds decreased the dehydrogenase activity in very low concentrations. Carbon disulfide, on the contrary showed the inhibiting action only when used in high concentrations (Table I). Sodium fluoride inactivates also the succinic dehydrogenase of vertebrates (Ammon et Dirscherl 1948).

The following paraffin and cycloparaffin anthelmintics inhibited the *Ascaris* dehydrogenase activity: diethyl ether (6.8×10^{-3} M), carbon tetrachloride (7.5×10^{-3} M), menthol (18.1×10^{-3} M), chloral hydrate (68.1×10^{-3} M). Chloroform showed no effect in concentrations examined (Table II), though, the inhibitory action of narcotics on dehydrogenases has long been proved. It was stated that urethane, i. e. ethyl carbamate, caused no dehydrogenase inhibition in low concentrations (Table II). It may be noted that in the above mentioned experiments of Lipschitz et Gottschalk 1921 — 4—15% urethane concentrations were used.

Aromatic and heterocyclic anthelmintics decreased also the succinic dehydrogenase activity, viz., rivanol, i. e. 2-ethoxy-6,9-diamino-acridine lactate (0.048×10^{-3} M), atebirin, i. e. 3-chloro-7-methoxy-9- (1-methyl-4-diethylamino-buthylamino) — acridine (0.74×10^{-3} M), thymol (0.96×10^{-3} M), betanaphthol (2.5×10^{-3} M), resorcin (7.5×10^{-3} M), piperazine adipate (11.2×10^{-3} M), guaiacol (14.2×10^{-3} M), salicylic acid (22.1×10^{-3} M), benzene (79.5×10^{-3} M). Pyridine, p-cymol and santonin had no effect in examined concentrations (Table III).

The following anthelmintic drugs (Table IV) inhibited the *Ascaris* dehydrogenase activity: Extractum Filicis siccum Riedel de Haën (0.0015%), male farn preparation Aspidium U.S.P. Penick and Company (0.0077%), Oleum Chenopodii (0.021%), Oleum Menthae piperitae F.P.III (0.043%), Oleum Terebinthinae rectificatum F.P.III (0.67%), Benzinum, i. e. petroleum benzin F.P.III (1.0%).

Table I
Effect of inorganic and metal-organic compounds on the succinic dehydrogenase activity of *Ascaris* in vitro in relation to control (100.0)

Concentration Compound	$10^{-1}M$		$10^{-2}M$		$10^{-3}M$		$10^{-4}M$		Concentration reducing activity to 50%
	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	
Sodium fluoride	17.6 ± 0.20	P<0.001	67.0 ± 0.71	P<0.01	98.1 ± 1.15	absent	100.0 ± 0.00	absent	$18.6 \times 10^{-3}M$
Potassium iodide	47.5 ± 1.30	P<0.001	98.7 ± 1.35	absent	100.0 ± 0.00	absent	101.9 ± 2.55	absent	$95.5 \times 10^{-3}M$
Boric acid	93.7 ± 1.04	P<0.01	98.3 ± 1.68	absent	103.3 ± 1.68	absent	102.0 ± 2.00	absent	not achieved
Carbon disulfide	insoluble	-	90.6 ± 0.61	P<0.01	100.0 ± 0.00	absent	98.8 ± 1.35	absent	not achieved
Aluminium sulphate	0.0 ± 0.00	P<0.001	30.9 ± 0.24	P<0.001	68.9 ± 1.00	P<0.01	98.2 ± 1.68	absent	$3.16 \times 10^{-3}M$
Mercuric chloride	-	-	-	-	-	-	0.0 ± 0.00	P<0.001	$0.27 \times 10^{-3}M$
Methiolate	-	-	0.0 ± 0.00	P<0.001	0.0 ± 0.00	P<0.001	0.0 ± 0.00	P<0.001	$5.23 \times 10^{-6}M$
Potassium antimonyl tartrate	38.0 ± 0.45	P<0.001	74.1 ± 0.71	P<0.001	89.5 ± 0.84	P<0.01	98.3 ± 1.68	absent	$45.0 \times 10^{-3}M$

Table II
Effect of paraffin and cycloparaffin compounds on the succinic dehydrogenase activity of *Ascaris* in vitro in relation to control (100.0)

Concentration Compound	$10^{-1}M$		$10^{-2}M$		$10^{-3}M$		$10^{-4}M$		Concentration reducing activity to 50%
	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	
Chloroform	insoluble	-	100.0 ± 0.00	absent	98.7 ± 1.35	absent	101.9 ± 2.55	absent	not achieved
Carbon tetrachloride 3)	insoluble	-	44.3 ± 1.00	P<0.01	100.0 ± 0.00	absent	98.7 ± 1.35	absent	$7.48 \times 10^{-3}M$
Ether	0.0 ± 0.00	P<0.001	22.4 ± 1.00	P<0.001	100.0 ± 0.00	absent	101.9 ± 2.55	absent	$6.84 \times 10^{-3}M$
Chloralhydrate	38.8 ± 1.00	P<0.001	100.0 ± 0.00	absent	100.0 ± 0.00	absent	100.0 ± 0.00	absent	$68.1 \times 10^{-3}M$
Urethane	100.0 ± 0.00	absent	98.7 ± 1.35	absent	100.0 ± 0.00	absent	-	-	not achieved
Urotropin	100.1 ± 1.29	absent	103.5 ± 1.68	absent	103.5 ± 1.68	absent	103.5 ± 1.68	absent	not achieved
Menthol 3)	insoluble	-	57.8 ± 0.66	P<0.01	99.4 ± 0.69	absent	102.8 ± 1.35	absent	$18.1 \times 10^{-3}M$

Explanation: 1) mean activity ± standard error, 2) significance of the difference, 3) in 2% ethanol.

Table III

Effect of aromatic and heterocyclic compounds on the succinic dehydrogenase activity of *Ascaris* in vitro in relation to control (100.0)

Concentration Compound	10 ⁻¹ M		10 ⁻² M		10 ⁻³ M		10 ⁻⁴ M		Concentration reducing activity to 50%
	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	Activity 1)	Signi- ficance 2)	
Benzene 3)	42.2 ± 1.42	P<0.001	98.7 ± 1.35	absent	101.4 ± 1.35	absent	100.0 ± 2.55	absent	79.5 × 10 ⁻³ M
p-cymol 3)	insoluble	-	insoluble	-	98.0 ± 0.89	P<0.01	100.0 ± 0.00	absent	not achieved
Thymol 3)	insoluble	-	0.0 ± 0.00	P<0.001	50.4 ± 0.24	P<0.01	98.0 ± 0.90	P<0.05	0.96 × 10 ⁻³ M
Salicylic acid 3)	insoluble	-	62.9 ± 0.63	P<0.001	101.4 ± 1.34	absent	101.9 ± 2.55	absent	22.1 × 10 ⁻³ M
Guaiacol 3)	0.0 ± 0.00	P<0.001	70.6 ± 1.14	P<0.01	102.1 ± 1.14	absent	102.1 ± 1.14	absent	14.2 × 10 ⁻³ M
Resorcin	0.0 ± 0.00	P<0.001	60.5 ± 1.00	P<0.001	93.8 ± 1.00	P<0.01	100.0 ± 0.00	absent	7.47 × 10 ⁻³ M
Betansaphol 3)	insoluble	-	59.0 ± 1.00	P<0.01	100.0 ± 0.00	absent	100.0 ± 0.00	absent	2.53 × 10 ⁻³ M
Santonin 3)	insoluble	-	insoluble	-	100.9 ± 0.59	absent	99.3 ± 0.69	absent	not achieved
Sulphathiazole	0.0 ± 0.00	P<0.001	74.3 ± 0.82	P<0.001	101.7 ± 1.67	absent	-	-	14.7 × 10 ⁻³ M
Atabrin	insoluble	-	0.0 ± 0.00	P<0.001	27.7 ± 0.26	P<0.01	100.0 ± 0.00	absent	0.74 × 10 ⁻³ M
Rivencol	insoluble	-	0.0 ± 0.00	P<0.001	0.0 ± 0.00	P<0.001	37.7 ± 0.61	P<0.01	0.048 × 10 ⁻³ M
Pyridine	98.7 ± 1.35	absent	98.7 ± 1.35	absent	100.0 ± 0.00	absent	100.0 ± 0.00	absent	not achieved
Piperazine adipate	0.0 ± 0.00	P<0.001	53.7 ± 0.54	P<0.01	100.8 ± 0.84	absent	101.5 ± 1.68	absent	11.2 × 10 ⁻³ M

Explanation: 1) mean activity ± standard error, 2) significance of the difference, 3) in 2% ethanol

Table IV

Effect of drugs of unknown chemical constitution or composition on the succinic dehydrogenase activity of *Asceris* in vitro in relation to control (100.0)

Drug	Oleum Chenopodii	Oleum Menthae pip. F.P. III	Oleum Terebinthinae rect. F.P. III	Extractum Filicis siccum	Aspidium U.S.F.	Argentum proteinicum F.P. III	Benzinum F.P. III
Concentration							
Activity 1)	1.68%	1.563%	1.0%	0.01%	0.1%	0.15%	1.0%
Significance 2)	0.0 ± 0.00 $P < 0.001$	0.0 ± 0.00 $P < 0.001$	0.0 ± 0.00 $P < 0.001$	0.0 ± 0.00 $P < 0.001$	0.0 ± 0.00 $P < 0.001$	0.0 ± 0.00 $P < 0.001$	54.7 ± 1.00 $P < 0.001$
Concentration							
Activity 1)	0.168%	0.156%	0.1%	0.001%	0.01%	0.015%	0.1%
Significance 2)	18.0 ± 0.21 $P < 0.001$	0.0 ± 0.00 $P < 0.001$	55.3 ± 0.51 $P < 0.001$	77.2 ± 1.12 $P < 0.001$	30.4 ± 5.00 $P < 0.01$	0.00 ± 0.00 $P < 0.001$	68.2 ± 0.71 $P < 0.001$
Concentration							
Activity 1)	0.0168%	0.0156%	0.01%	0.0001%	0.001%	0.0015%	0.01%
Significance 2)	47.6 ± 0.36 $P < 0.001$	98.4 ± 1.21 absent	91.2 ± 1.00 $P < 0.01$	99.0 ± 1.26 absent	98.7 ± 1.35 absent	34.5 ± 0.35 $P < 0.001$	100.1 ± 2.39 absent
Concentration							
Activity 1)	0.0017%	0.0016%	0.001%	-	0.00001%	0.00015%	-
Significance 2)	86.3 ± 1.34 $P < 0.01$	100.0 ± 0.00 absent	101.4 ± 1.35 absent	-	100.0 ± 0.00 absent	100.1 ± 1.29 absent	-
Concentration reducing activity to 50%	0.0208%	0.043%	0.67%	0.0015%	0.0077%	0.00150%	1.04%

Explanation: 1) mean activity $\pm$ standard error, 2) significance of the difference, 3) in 2% ethanol.

Among the parasiticide drugs not used in helminthic infections sulphathiazole ($14.7 \times 10^{-3}M$) and protein silver, i. e. Argentum proteanicum F.P.III (0.0015%), inactivated the *Ascaris* dehydrogenase, boric acid and urotropin had no effect (Table II).

It may be seen that out of the 31 examined anthelmintics 26 inhibited the succinic dehydrogenase activity of *Ascaris lumbricoides* in concentrations of the order of $10^{-2}M$ — $10^{-3}M$, or 0.1% — 0.001%. It must be emphasized that this effect was stated, though, the cytochrome oxidase (the most poison sensitive part of the succinic oxidase system, Quastel) is not present in *Ascaris* muscle. Therefore, the inhibition of the *Ascaris* succinic dehydrogenase, the enzyme of great importance to the endoparasitic organisms living in essentially anaerobic environment, seemed likely to take part in the anthelmintic action of the drugs. It may be assumed that the evaluation of the inhibitory effect of the compounds on *Ascaris* dehydrogenase may be of use in new anthelmintics research.

Summary

The following anthelmintics inhibit the succinic dehydrogenase activity of *Ascaris lumbricoides* f. suis: sodium fluoride, potassium iodide, carbon disulphide, aluminium sulphate, mercuric chloride, merthiolate, potassium antimonyl tartrate (Table I); carbon tetrachloride, ether, chloral hydrate, menthol (Table II); benzene, p-cymol, thymol, salicylic acid, guaiacol, resorcin, betanaphthol, atebrin, rivanol, piperazine adipate (Table III); Ol. Chenopodii, Ol. Menthae pip., Ol. Terebinthinae rect., Extr. Filicis siccum, Aspidium, petroleum benzin (Table IV).

Chloroform, pyridine and santonin have no inhibitory effect (Table II, III).

It is assumed that the *Ascaris* dehydrogenase inhibition may take part in the anthelmintic action of the drugs.

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STRESZCZENIE

Zbadano zmodyfikowanym sposobem Bertha działanie 31 leków przeciwo-robaczych in vitro na aktywność dehydrogenazy kwasu bursztynowego glisty *Ascaris lumbricoides* f. *suis*. Preparat dehydrogenazy otrzymywano sposobem Keilina i Hartree z woru skórno-mięśniowego glisty. Oznaczenia wykonywano w następujących warunkach: błękit metylenowy $5 \times 10^{-5}M$, bursztynian sodu $3 \times 10^{-3}M$, bufor fosforanowy o pH 7,4 około $36,7 \times 10^{-3}M$, temperatura 38° przy wahaniach $0,5^{\circ}$. Odbarwienie błękitu metylenowego przeprowadzono w 90% oznaczając przez porównanie z wzorcem zawierającym 10% barwnika. Czas odbarwienia prób kontrolnych 18 — 27 min., średnie odchylenie aktywności prób kontrolnych przyjętych za 100 s = $\pm 1,64$, średni błąd e = $\pm 0,193$. Stężenie badanych związków 10^{-1} — $10^{-4}M$ w miarę możliwości uzyskania tych stężeń lub 1,68% — 0,0001%. Posługując się równaniem regresji, obliczono stężenie leku obniżające aktywność dehydrogenazy o 50%.

Znamienne statystyczne obniżenie aktywności dehydrogenazy kwasu bursztynowego glisty wywołały liczne związki w stężeniach podanych w ostatniej rubryce tabel I — IV. Nie wykazały obniżenia aktywności dehydrogenazy w badanych stężeniach: chloroform, pirydyna, santonina (tabele II i III).